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Experimental Studies on the Effects of Heated Chrysotile Asbestos and Automobile Brake Lining Dust Injected into the Body Cavities of Mice[1]

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A series of experiments with mice involving the intrapleural injection of chrysotile asbestos dust heated to varying temperatures up to 1000°C suggests that the physical shape of the dust particles is more important than the altered chemistry of the dust. Chrysotile heated to no more than 400°C produced dust samples containing many long fibers, and these samples resulted in large pleural granulomas. Chrysotile heated to more than 400°C produced dust containing mainly particles of low aspect ratio, and these produced very small granulomas. The initial size of the granulomas was very closely related to the final degree of fibrosis found in the animals and, therefore, chrysotile heated to 400°C or less was much more fibrogenic than when it was heated to higher temperatures. A sample of automobile brake lining dust was found to contain very little recognizable chrysotile, and consisted mainly of particles with a low aspect ratio. When this dust was injected into the pleural cavities of mice it produced very small granulomas and little fibrosis.

The discovery by Wagner *et al.* (1960) that exposure to asbestos dust was often associated with the production of mesothelial tumors caused concern about the possible pollution of the normal urban atmosphere by asbestos dust. Wagner had shown that very small doses were apparently sufficient to produce tumors in some cases and it was suggested that the use of asbestos products from ironing boards to brake linings might be liberating sufficient dust into the atmosphere to cause a health hazard. Since automobile brake linings are constantly being abraded in use and since many tons of asbestos are consumed in this manner each year, the possibility of atmosphere pollution from brake lining dust has received particular attention.

In order to test the level of atmospheric pollution by asbestos a number of studies have been undertaken. The lungs from unselected autopsy cases from different parts of the world have been examined and initially, since uncoated asbestos dust is difficult to find in tissues, the presence of asbestos bodies was used as a likely index of the presence of asbestos. The figures obtained showed an increase in the percentage of cases containing bodies with the increasing sophistication of the techniques used. The first of these studies was that undertaken by

[1] The dust samples used in this study were prepared in the laboratories of T.B.A. Industrial Products Ltd. of Rochdale, England. This work was undertaken as part of the research programe organised by the British Asbestosis Research Council.

Thomson *et al.* (1963) in Capetown and these workers reported bodies in more than 30% of cases. Later, Thomson and Graves (1966) found a similar prevalence in Miami; Cauna, Totten, and Gross (1965) found bodies in 40% of a series of lungs from Pittsburg and Anjilvel and Thurlbeck (1966) found them in 45% of their cases in Montreal. Meurman (1966) reported finding bodies in 70% of a group of lungs from Finland and finally, Utidjian, Gross, and De Treville (1968) using a new technique, reported that the lungs of 97 out of 100 consecutive autopsies from Pittsburg contained bodies. However, Davis, Gross, and De Treville (1970) showed that the production of ferruginous bodies was not specific for asbestos as had been previously believed and it therefore became necessary to undertake new studies by which asbestos could be identified by precise methods. This is still a very difficult problem, although Langer *et al.* (1970) and Pooley *et al.* (1970) have shown that chrysotile asbestos can be positively identified in a large proportion of the lungs of town dwellers.

The initial concern over generalized atmospheric pollution by asbestos involved the possibility of tumor production but the problem was given a new dimension by the work of Jagatic (1967). Jagatic reported that chrysotile heated to high temperatures was acutely toxic to mice and found that after the injection of heated chrysotile into the peritoneal cavity, 60% of mice died within 48 hr. Since chrysotile is the type of asbestos usually used in brake linings and since, during braking, the linings are heated to very high temperatures, it became necessary to consider the possible acute hazards of the inhalation of brake lining dust.

In order to study the biological effects of heated chrysotile and brake lining dust in more detail it was decided to undertake a series of experiments in which samples of these dusts were injected into the pleural and peritoneal cavities of mice. In later studies it is hoped to undertake inhalation experiments with these minerals to see whether they should be considered as dangerous atmospheric pollutants.

MATERIALS AND METHODS

The mineral samples used in these studies were prepared as follows:

Samples of high grade Cassiar chrysotile asbestos were heated in an electric tube furnace for 4 hr at temperatures of either 400°C, 600°C, 800°C, or 1,000°C. After cooling, the chrysotile was ground in a mechanical mortar and pestle and half of each sample was sieved through a 250-mesh copper sieve. With normal chrysotile and chrysotile heated to no more than 400°C many long fibers remained in the dust after grinding and since most of these were removed by sieving it was possible to produce samples both with and without long fibers. With chrysotile heated to more than 800°C, however, the dust fibers became extremely brittle and grinding was found to break most of these into relatively short lengths. With these samples, therefore, sieving made little difference to the size distribution of the dust particles although experiments were conducted with both sieved and unsieved samples to test their effects on tissues.

The thermal decomposition of chrysotile follows a two-stage sequence of dehydroxylation and breakdown, and the mechanism has been studied by several authors including Ball and Taylor (1963) and Brindley and Hayami (1965). The dehydroxylation of chrysotile takes place in the temperature range 600–780°C and

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800–850°C the dehydroxylated noncrystalline residue is said to recrystallize to give forsterite and silica. The total decomposition corresponds to

$$Mg_6\ Si_4\ O_{10}\ (OH)_8 = 3Mg_2\ SiO_4 + SiO_2 + 4H_2O\ .$$

The forsterite persists together with silica up to 1000°C but above this temperature some enstatite is formed

$$3Mg_2\ SiO_4 + SiO_2 = 2Mg_2\ SiO_4 + Mg_2\ Si_2\ O_6\ .$$

Since the highest temperature used in these experiments was 1000°C it was the decomposition of chrysotile to forsterite and silica that formed the basis of the present study. Throughout this paper the dust samples are referred to as heated chrysotile for simplicity. Readers interested in the exact chemistry of any particular sample are referred to the information in this paragraph.

The sample of brake lining dust used in these experiments was supplied by a commercial producer of brake linings and had been taken from the rear brake drums of two of their test vehicles. These cars had been put through a test routine designed to simulate a long period of normal usage. The original brake lining composition was similar to that described by Lynch in 1968 and in addition to chrysotile asbestos and binding resins the linings had contained both chromium oxide and metallic aluminium granules. It would normally be expected that brake lining dust would contain metallic iron from the brake drum, but an X-ray analysis of the dust sample performed by A. L. Rickards failed to detect crystalline iron on this occasion.

In these samples of heated chrysotile and brake lining dusts the following experiments were undertaken. In the first series, in order to confirm the results of Jagatic *et al.* (1967), doses of 250 mg of chrysotile heated to 1000°C were injected into the peritoneal cavities of 25 Balb/C mice. In the second series of experiments single 10 mg doses of normal chrysotile and chrysotile heated to either 400°C, 600°C, 800°C, or 1000°C were injected into the pleural cavities of similar mice. Batches of 25 mice were used for each dust sample and both sieved and unsieved dust specimens were used in each case. In the final series of experiments single doses of 10 mg of automobile brake lining dust were injected into the pleural cavities of another batch of 25 Balb/C mice. In all cases the dust samples were suspended in 1 ml of sterile distilled water before injection. Animals from all these studies were killed at intervals from 7 days to 1 yr after injection and samples of tissue from each were fixed in either formol saline for light microscopy examination or buffered osmium tetroxide for electron microscopy. For light microscopy, sections were stained with either hematoxylin and eosin, E.P.S., or Perls' stain for iron. For electron microscopy, the tissues were embedded in araldite and after sectioning were stained with lead citrate.

OBSERVATIONS

In the first series of experiments those animals given an intraabdominal injection of 250 mg of chrysotile heated to 1000°C showed signs of toxic effects within 24 hr and by 48 hr seven had died. The remainder showed continuing signs of disability for several days but all eventually recovered and none died within 6 months of injection. When those mice that had died within 48 hr of injection were

examined it was found that little or no cellular response had developed around the dust which remained scattered and loose in the peritoneal cavity. A histological examination of the viscera of those animals showed no signs of damage with the exception of some patchy hydropic change in the kidney tubules. When mice from this series were killed 7 days after dust injection it was found that the dust in the peritoneal cavity had by now become compacted into large granulomas. Most of these remained free in the peritoneal cavity, supported by strands of connective tissues, but some became attached to the surface of the omentum. It was only occasionally however that adhesions were formed between the loops of the intestine. Within the granulomas the cellular reaction to the dust consisted largely of macrophages with a few giant cells, fibroblasts, lymphocytes, and plasma cells and with the smaller lesions cells were able to penetrate throughout the dust mass. With a dose of 250 mg, however, some of the dust lesions were so large that complete penetration did not occur and the central regions of these granulomas remained completely acellular. By 2 wk after dust injection most of the granulomas were surrounded by a thin but clearly defined capsule of fibrous tissue and some collagen had been produced among the cells of the granuloma itself. A gradual increase in collagen was seen within the lesions between 7 days and 1 yr after injection but within this period the granulomas still contained many cells.

In the experiments using 10 mg doses of either heated chrysotile or brake lining t no animals died within the first 2 wk after injection and none showed any toxic effects. Granulomas were produced in response to all the injected samples but cellular reaction to the dust was found to vary according to the dust type, the temperature to which it had been heated, and its method of preparation. Animals injected with either normal chrysotile or chrysotile heated to 400°C were found after 2 wk to have cellular granulomas with complete cellular penetration throughout the dust masses. In these lesions cells masked the dust to such an extent that it was extremely difficult to see with the light microscope. In the animals injected with chrysotile ground and sieved the granulomas were relatively small and formed few adhesions, but in those animals injected with unsieved dust the granulomas were much larger for the same dust dose and frequently formed adhesions between the contents of the chest cavity. In animals injected with dust heated to 600°C cellular granulomas were still produced but there appeared to be less difference between the sieved and the unsieved dust groups, and both produced only small granulomas without adhesions. In the groups of animals injected with chrysotile heated to either 800 or 1000°C the resulting granulomas were always small compared with the dust dose used and no adhesions were found in either the sieved or the unsieved group. In these experiments, however, it was found that the chrysotile dust was now clearly visible in the tissues and in the light microscope appeared to consist of cylindrical or rectangular particles which ranged from 1 to 10μm in diameter and which could be up to 50μm in length. Normally cells were still able to penetrate throughout the dust masses but fewer cells were present than in granulomas produced by normal chrysotile.

By 2 wk after dust injection a thin capsule of fibrous tissue was usually formed und those granulomas remaining free in the pleural cavity. Those that formed adhesions, however, did not develop any specific layer of fibrous tissue between the main granuloma and the organ to which they were attached. By 2 wk, also

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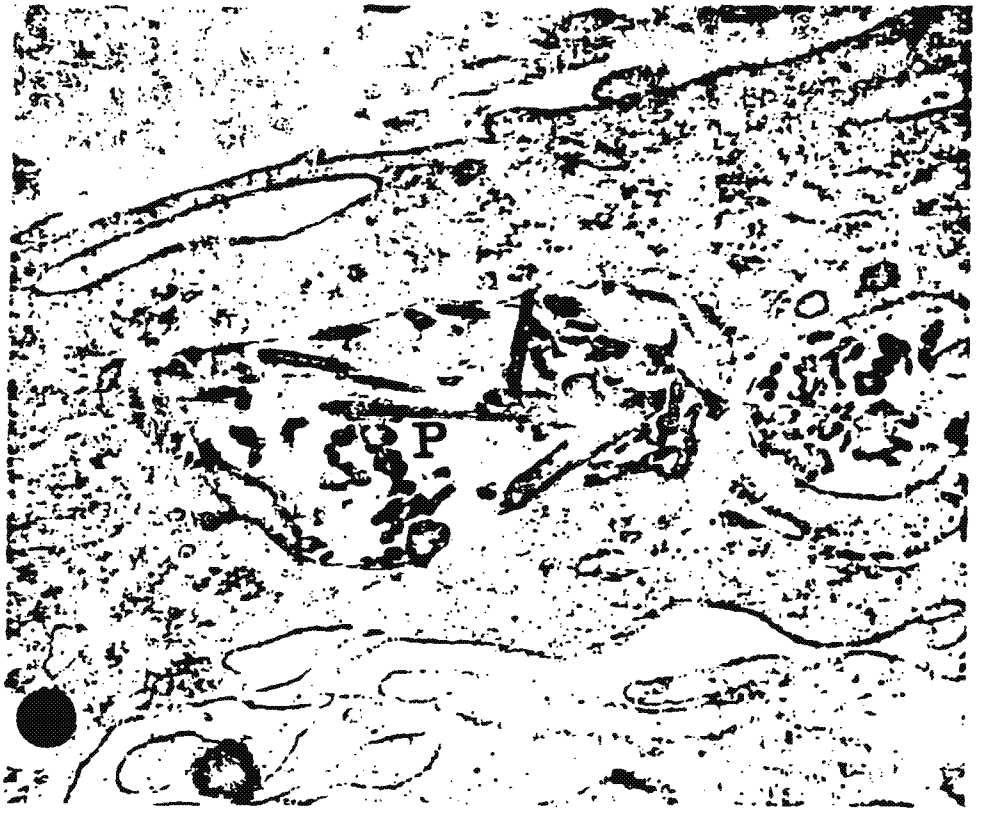

FIG. 1. A section of a macrophage from a mouse granuloma 2 wk after an injection of chrysotile asbestos heated to 600°C. Groups of chrysotile crystals are seen in two phagosomes (P) within the cell, and the structure of these crystals appears normal. In both cases, the dust appears loosely arranged in the phagosomes and the phagosome membrane has not at this stage contracted closely around the dust. × 65,000.

ome collagen was usually present among the plagocytic cells that made up the nain part of the lesions. During the first few weeks after injection collagen production was more rapid in those granulomas induced by chrysotile dust heated o 800°C or more, than in granulomas resulting from either unheated chrysotile or chrysotile heated to no more than 600°C. However, by 6 mo after injection this distinction was no longer apparent, and the long-term production of collagen in any given area of granulation tissue did not appear to differ much between imilarly prepared chrysotile samples that had been heated to different temperatures. The physical shape of the dust particles did, however, have considerable effect on fibrosis. The total amount of collagen produced in response to a 10-mg lose was closely related to the size of the resulting granulomas and since unsieved samples heated to no more than 400°C produced large lesions these samples were more fibrogenic than sieved samples heated to 400°C or to samples heated to igher temperatures. All these produced very small granulomas.

Electron microscope studies showed that almost all the injected dust from heated at 600°C or less was phagocytosed by macrophages and occasional ...t cells and few extracellular dust particles were found. The process of

FIG. 2. A section of a mouse granuloma 1 wk after the intrapleural injection of chrysotile dust heated to 800°C. A large bundle of fused chrysotile crystals has been phagocytosed by a macrophage (M), and the phagosome membrane is in close contact with the dust (arrowed). Although the chrysotile crystals are partially fused with one another, the original crystal outline is still discernible. × 65,000.

phagocytosis and the subsequent behavior of the dust within cells appeared identical to that reported previously (Davis, 1968, 1970a). Dust particles were initially taken up into large vacuolated phagosomes (Fig. 1), but the membranes of these eventually contracted and became closely opposed to the dust particles. Usually the dust remained on its own inside the phagosome membrane, but occasionally it was found mixed with ferruginous granules and membrane debris in the dense structures that are known as phagosome residual bodies. Most of the dust remained in those structures, but occasionally an odd fiber was found that appeared to have escaped into the cell cytoplasm. The macrophages from these experimental groups showed no signs of damage even when they contained large amounts of dust. No differences could be detected in the structural appearance of crystals of normal chrysotile and chrysotile crystals that had been heated to 400°C. With dust heated to 600°C, however, it was noticeable that the phagocytosed dust, whether sieved or unsieved, contained more individual chrysotile crystals than crystal bundles, and many phagosomes were found closely packed with individual crystals. Electron microscope examination of lesions produced by chrysotile heated to 800°C or 1000°C showed why the dust was clearly visible in these lesions even with the light microscope. During the heating process the individual

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el tile crystals had lost their normal tubular structure and fused with the other c stals in the bundle (Rickards, 1967). This fusion was complete enough to hold the crystals together during grinding so that the dust was broken up into relatively a e masses rather than fine fibrous bundles often containing only a few crystals h. When the tissues were examined only a few weeks after dust injection the original outline of the chrysotile crystals within the fused masses could still be detected (Fig. 2), but several months after dust injection this crystalline structure was no longer visible. It appeared that the dust had undergone some degree of dissolution within the tissues, and the neat crystal arrangement had been replaced by an irregular honeycomb structure (Fig. 3). Although the fused chrysotile masses were much thicker than normal chrysotile dust particles, most of them had no greater length, and the majority could still be phagocytosed by single macrophages. Very large particles were occasionally seen inside giant cells although these cells are not common in mouse granulomas (Davis, 1970b), and more frequently the larger particles were closely surrounded by unfused macrophages. Inside the cells there were some notable differences between the behavior of the heated chrysotile masses and normal chrysotile. Particles of normal chrysotile are taken up into large phagosomes which presumably contain fluid and the phagosome membrane is often well clear of the contained dust, at

3. A section of a macrophage from a mouse granuloma 3 mo after the injection of chrysotile heated to 800°C. The macrophage is surrounded by collagen fibers (F), but a large of fused chrysotile (C) is present within the cytoplasm. In this case, however, the outline has been lost and has been replaced by an irregular honeycomb pattern.

FIG. 4. Particles of chrysotile dust within a macrophage from a mouse pleural granuloma, 3 mo after dust injection. The dust had been heated to 800 C before injection, and by this stage the chrysotile crystal pattern has been replaced by an irregular honeycomb structure. The largest dust particle (P) is associated with masses of dense granules (F) that probably represent ferritin, or haemosiderin, and in some areas these have penetrated deeply into the dust. × 35,000.

least in the early stages. With the masses of heated fused chrysotile however, although they were taken up into membrane bounded vesicles the membrane was always seen in close contact with the dust. Many of the small dust masses could still be taken up by single macrophages which usually showed no signs of damage. In these granulomas, however, it was not uncommon to find dead macrophages containing dust although such dead macrophages have only rarely been found in lesions produced by normal chrysotile.

In animals injected with either unsieved normal chrysotile, or chrysotile heated to 400°C some typical ferruginous bodies (Davis, 1970b) were found in the granulomas from about 2 wk after dust injection. Sieved samples of normal chrysotile and chrysotile heated to 400°C did not, however, result in the production of any ferruginous bodies. This was also true of both sieved and unsieved samples of chrysotile heated to 600°C. In animals treated with chrysotile heated to either 800°C or 1000°C no true asbestos bodies were found in which the dust particles were surrounded by a distinct coating of ferruginous material. However, with some of the larger chrysotile fragments, especially those showing the greatest degree of dissolution, it appeared that dense granules similar to ferritin or haemosiderin had penetrated deep into the dust (Fig. 4).

Brake lining dust when injected into the pleural cavity of mice produced smaller granulomas than any of the asbestos types used in this study and these

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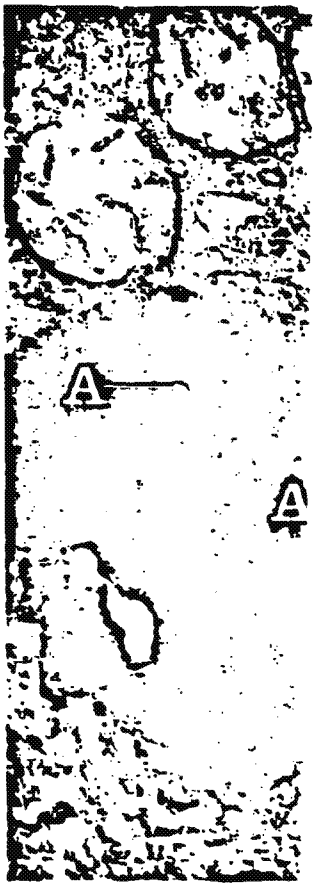

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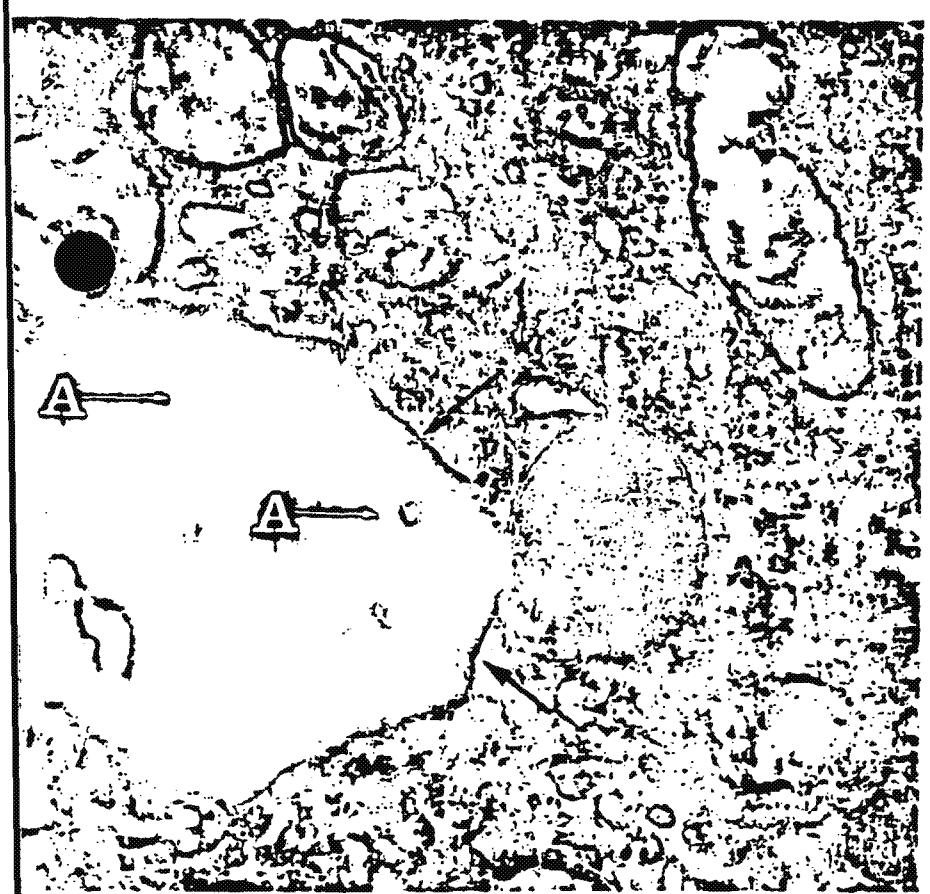
...mas never formed adhesions, being mainly suspended in the pleural cavity ...in strands of connective tissue. In the light microscope the brake lining dust ...uld be seen to consist mainly of irregularly shaped particles 5–25 μm in ...meter, but a number of elongated particles were also present which ranged ... 2 to 25 μm in diameter and which could be up to 50 μm in length. Cells ...re able to penetrate deeply into the dust masses but the number of cells was ...ways comparatively few compared to the dust load. The granulomas were ...dly surrounded by a thin capsule of fibrous tissue within a few days of ...ction, and some collagen had been produced among the dust containing cells ...thin 2 wk. After this time collagen production increased quite rapidly and the ...is became less cellular so that 1 yr after injection the dust particles had ... bound together by a network of old acellular collagen. Since the initial ...nulomas were so small, however, the amount of collagen produced in response ...t dust was never very great.



5. A particle of automobile brake lining dust within a macrophage from a mouse pleural ...oma 2 wk after dust injection. The dust is separated from the cell cytoplasma by a ...rane, but this is closely opposed to the dust at al points (arrowed). The dust consists ... of dense amorphous material, and contains no recognizable chrysotile asbestos. There ...within the dust some dense crystalline particles that probably represent metallic ... × 60 000.

Electron microscope studies showed that the brake lining dust consisted mainly of dark masses showing no internal structure, and containing no recognizable chrysotile. In most cases the only contaminants within the dust masses were smooth crystalline particles usually about 0.05 of a micron in diameter (Figs. 5 and 6). Since metallic iron was not present in the original dust sample these were probably particles of metallic aluminum added to the original brake lining mixture as an abrasive. Very occasionally dust particles were found that consisted of a lighter and more homogenous material which contained in addition to the crystalline particles already mentioned elongated crystals of apparently normal chrysotile (Fig. 7). This type of dust, however, made up much less than 1% of the total. Small dust particles were phagocytosed in large numbers by single macrophages but as with chrysotile heated to high temperatures normal phagosome vacuoles were not seen even in specimens examined only a few days after dust injection. The phagosome membrane contracted quickly around these smaller dust particles to form phagosome residual bodies, and these often contained some ferruginous granules and membrane debris as well as dust (Fig. 6). The larger dust fragments were sometimes found within small giant cells, but as with heated

FIG. 6. A macrophage from a mouse pleural granuloma 4 wk after the injection of brake lining dust. Dust particles (D) are present, with membrane elements and some fine granular material, in irregular structures that probably represent phagosome residual bodies. × 46 000.

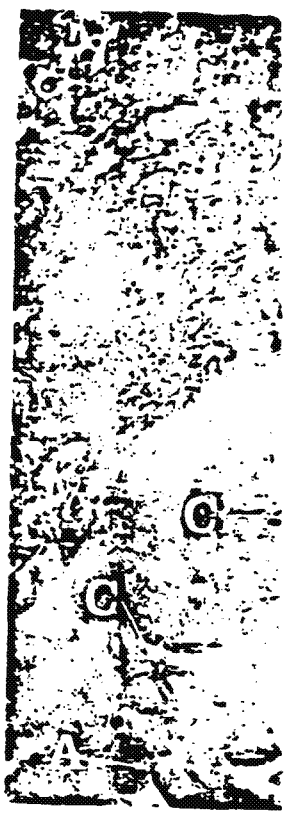


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FIG. . A particle of brake lining dust within a mouse macrophage 2 wk after the injection this dust. In this instance the dust consists of low density amorphous material within which ubedded recognizable crystals of chrysotile asbestos (C) and larger nonfibrous crystalline les that probably represent metallic aluminum (A). × 63,000.

sotile many of the larger particles although surrounded by cells, remained cellular. Dead macrophages containing brake lining dust were found in the nulomas at all stages of the experiment, but they were less frequent than in ulomas produced by chrysotile heated to either 800°C or 1000°C.

DISCUSSION

e present study has confirmed previous suggestions that the physical shape bestos particles is important in determining the degree of fibrosis produced ssues. Several workers including King *et al.* (1946), Vorwald *et al.* (1951), Webster (1965) have suggested that long fiber asbestos is more fibrogenic g tissue than short fiber dust and Davis in 1972 showed that this also applied lust injected into the pleural cavity. It was found in this work that long fiber les of a number of mineral dusts produced much more fibrosis than short dust. In the studies using heated chrysotile it was found that if the dust was ted to no more than 400°C mechanical grinding still produced dust containe large proportion of long fibers. When normal chrysotile is finely ground riginal crystal bundles tend to split longitudinally rather than transversely re very long thin fibers are produced. Sieving this dust removes most

of these long fibers and it was therefore possible by using both sieved and unsieved dust samples to gain some idea of the effect of fiber length on the tissues. It was found that the long fiber samples produced larger granulomas than the short fiber dust and since the amount of fibrous tissues produced depended on the initial size of the lesions (Davis 1972) long fiber dust was more fibrogenic than short.

When dust was heated to 600°C the structure of the chrysotile crystals appeared unchanged in the electron microscope but the ground dust samples contained few long fibers. It appeared that the crystals were more brittle than normal chrysotile and that in most cases the bonding between the crystals had been weakened. This was probably associated with the gradual dehydration that occurs when crysotile is heated from between 100°C and 600°C. Grinding this type of dust tended to break it into individual short crystals and there was little difference between sieved and unsieved dusts. Since few long fibers were present both samples resulted in the production of small granulomas and little fibrous tissue was produced compared to the size of the dust dose. When chrysotile was heated to 800°C or 1000°C the individual crystals fused with one another and the fused bundles remained brittle. When these samples were ground the original large bundles of chrysotile crystals fractured transversely as one unit and the resulting dust consisted of thick, but short particles. Sieving this dust did not change the particle size distribution and both samples produced very small granulomas resulting in relatively little fibrosis. With the brake lining dust the particle size distribution was very similar to chrysotile heated to 800°C or more. Some enlongated particles were present but these always had a very low aspect ratio. Since very little of this dust could be recognized as chrysotile even with the electron microscope, the results are not directly comparable with heated chrysotile but very small granulomas were produced in response to brake lining dust and little fibrosis resulted from its injection.

The first group of experiments in this study confirmed the suggestions of Jagatic *et al.* (1967) that chrysotile asbestos heated to high temperatures is far more toxic than normal chysotile. It would appear, however, that very high doses are needed to produce a lethal effect and after a 10 mg dose none of the mice showed any signs of disability. This 10 mg dose for mice corresponds to one of approximately 30 g for a 12 stone man and this is an impossible figure for anyone to take in by inhalation over a short period even under the worst industrial conditions. It is very unlikely, therefore, that this toxic effect of heated chrysotile need be considered seriously when dealing with the problems of general atmosphere pollution by this type of asbestos. From the experimental point of view, however, the reasons for this toxicity are of considerable interest. It has been suggested that when chrysotile is heated to more than 800°C free silica is liberated in the amorphous state, and several workers have shown that this can be lethal. Gye and Purdy (1922) demonstrated that colloidal silica was rapidly fatal to experimental animals when injected intravenously and Dale and King (1953) produced similar results. These workers found that colloidal silica was ten times more toxic than crystalline quartz in these conditions. Klosterkotter and Otten (1953) also showed that molecularly dispersed silica, whether administered intracheally or intraperitoneally, was lethal to rats. It therefore seems possible that

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response to the dust and

aths of animals given large injections of heated chrysotile were due to silica poisoning.

egardless of general systemic toxicity, however, it was found that chrysotile eated to 800°C or more and brake lining dust both showed much more cytotoxicity for individual macrophages than normal chrysotile. In a previous study sing the intrapleural injection of chrysotile in experimental animals (Davis, 970a) it was noted that it was very rare to find dead macrophages in the lesions. With heated chrysotile or brake lining dust, however, macrophage death was quite frequent although dead macrophages never made up more than a few ercent of the total. Even the living macrophages from these granulomas showed distinct differences from those in lesions produced in response to normal chrysotile. When normal chrysotile is phagocytosed by macrophages it is retained r some time in a large phagosome vacuole before the membrane of this structure contracts around the dust particle. It was shown in organ culture experiments (Davis, 1968) that this process could be completed in as little as 12 hr after hagocytosis but this must be rare and after intrapleural injection of chrysotile acrophages with vacuolated dust containing phagosomes could be found in the granluomas for several weeks. With chrysotile heated to 800°C or more, however, d brake lining dust, vacuolated phagosomes were never seen and the phagosome embrane was always closely opposed to the dust particle. From these findings it would appear that chemical differences between the dust samples are responsible for quicker contraction of the phagosome membrane and the formation of sidual bodies, and it is interesting to consider whether this property was related to the raised content of amorphus silica in the heated samples.

Marks *et al.* (1956) showed that whereas amorphous silica produces the greatest degree of systemic toxicity, the crystalline varieties are more rapidly cytotoxic to macrophages. It may be, however, that while noncrystalline silica is unable to cause the rupture of phagosome membranes (Allison *et al.*, 1965) it is nonetheless able to increase the permeability of most phagosome membranes enough to reduce phagosome contraction time.

The finding that chrysotile heated to more than 600°C is more cytotoxic than normal chrysotile yet results in less fibrosis appears to contradict Heppleston's 1964 finding that macrophage death is an essential precursor to fibrosis resulting from silica dust. In fact, however, two conflicting factors are probably involved in these findings. It has been found (Davis, 1972) that the amount of fibrosis resulting from the injection of mineral dusts closely corresponds to the size of the initial cellular response to the dust and this in turn depends largely on the fiber length. Since the heated chrysotile contained no long fibers the resulting granlomas were very small and although macrophage death resulting from increased dust cytotoxicity probably accelerated the production of collagen in these lesions the total collagen production was never very large.

The finding that automobile brake lining dust has a very low fibrogenicity is encouraging, but the initial concern over the pollution of the atmosphere by brake lining dust concerned its possible association with the production of mesotheliomas, and the present study has not settled this point with certainty. No tumors were found in animals injected with either heated chrysotile or brake lining dust but the experiments were mainly concerned with the initial tissue response to the dust and were terminated after 1 yr. It is now known, however,

that whereas intrapleural injection of asbestos in rats produces large numbers of mesotheliomas (Wagner, 1960) few are produced by similar injection in mice (Davis, unpublished) and so no significant results would have been produced from prolonged experiments of this type using brake lining dust. It is planned to undertake new series of experiments that will test the long-term effects of brake lining dust in conditions where normal chrysotile is known to produce tumors. At this point, however, it may be pointed out that electron microscope studies of lesions produced in response to brake lining dust have shown that this dust contains extremely little recognizable chrysotile and what there is is mostly embedded in the plastic bonding material. No computation of urban atmospheric pollution by chrysotile from this source can therefore be made by calculating brake drum wear.

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